Journal of Taibah University Medical Sciences (2016) 11(5), 469–477

Taibah University

Journal of Taibah University Medical Sciences

www.sciencedirect.com

Original Article

Characterization of tannic acid- and gallic acid-functionalized single- and multiwalled carbon nanotubes and an *in vitro* evaluation of their antioxidant properties

Krishnamoorthy Rajavel, PhD [a], Rajkumar Gomathi, PhD [b], Sellamuthu Manian, PhD [c] and Ramasamy Thangavelu Rajendra Kumar, PhD [d,*]

[a] *Advanced Materials and Devices Laboratory, Department Physics, School of Physical Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India*
[b] *Laboratory of Flavor and Chromatographic Analysis, Federal University of Sergipe, SãoCristóvão, SE, Brazil*
[c] *School of Life Sciences, Department of Botany, Bharathiar University, Coimbatore, Tamil Nadu, India*
[d] *Department of Nanoscience and Technology, School of Physical Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India*

Received 30 March 2016; revised 12 July 2016; accepted 31 July 2016; Available online 4 September 2016

الملخص

أهداف البحث: الأنابيب النانوية الكربونية هي مؤكسدات قوية تؤثر على تطبيقاتها الطبية الحيوية. ويتأثر دورها الفاعل كمضادات للأكسدة بوظيفتيها، كمثبط للسمية وكمحفز للتأثيرات المضادة للأكسدة. تختص هذه الدراسة بالقدرة الكامنة للأكسدة لكل من الأنابيب النانوية الكربونية أحادية الجدار والأنابيب النانوية الكربونية متعددة الجدر بتفعيلهما بواسطة حمض التانيك وحمض القاليك وتقييم خصائصهم المضادة للأكسدة مخبريا.

طرق البحث: تمت عملية التفعيل بواسطة جهاز "فوريه" لتحويل طيف الأشعة تحت الحمراء وتقييم المحتوى الفينولي الكلي. أما تحليل مضادات الأكسدة فتم بواسطة كل من كسح أيمينو أزينيوم-ثنائي الفينول- (٦.٤.٢) ثلاثي نايترات الفينول) وأكسدة الدهون بالبيروكسيد، والتحديد الكمي لأنواع الأكسجين التفاعلي وإخماد الهيدروكسيل والجذور فائقة الأكسدة المتولدة خارجيا.

النتائج: يتوافق توظيف مضادات الأكسدة على الأنابيب النانوية بواسطة جهاز "فوريه" لتحويل طيف الأشعة تحت الحمراء وتقييم المحتوى الفينولي الكلي. لوحظ كسح الجذور العليا بالنسبة للأنابيب النانوية الكربونية أحادية الجدار المفعلة بواسطة حمض التانيك مقارنة بغيره من التفعيلات، وكذلك بالأنابيب النانوية الكربونية متعددة الجدر. أظهرت نتائج أكسدة الدهون بواسطة البيروكسيد أن توظيف الأنابيب النانوية بمضادات أكسدة حمض التانيك خفضت أكسدة الدهون بشكل ملحوظ (٣٦٪) مقارنة بالثابت المتعري (٨٥٪) والثابت الإيجابي (٩٤٪). إضافة إلى ذلك فإن الأنابيب النانوية المفعلة بواسطة مضادات الأكسدة أظهرت إنتاجا لا يُذكر من أنواع الأكسجين التفاعلي تحت ظروف مختلفة من الإشعاع وجذور الهيدروكسيل وفائقة الأكسدة المتولدة خارجيا.

الاستنتاجات: أوضحت هذه الدراسة أنه باستخدام النماذج المخبرية فإن التوظيف الفاعل للأنابيب النانوية الكربونية باستخدام حمض التانيك وحمض القاليك يؤدي إلى خصائص مضادة للأكسدة مذهلة. وأبدت الأنابيب النانوية الكربونية المفعلة كمضادات للأكسدة انخفاضا في نسبة موت الخلايا مصحوبا بإنتاج لا يُذكر من أنواع الأكسجين التفاعلي بتأثير الحالات الإشعاعية المختلفة، وانخفاضا في جذور الهيدروكسيل وفائقة الأكسدة المتولدة خارجيا. إضافة إلى ذلك فإن الأنابيب النانوية الكربونية المفعلة كمضادات للأكسدة كان لديها توافقا أكبر مع جدار الخلية.

الكلمات المفتاحية: مضادات الأكسدة؛ التوافق مع الحياة؛ الأنابيب النانوية الكربونية؛ التوظيف؛ الكسح الجذري

Abstract

Objectives: Carbon nanotubes (CNTs) have powerful oxidative properties that influence their biomedical applications. This study addresses the oxidative potential of both single-walled carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes (MWCNTs) by functionalizing them with tannic acid (TA) and gallic acid (GA), and an *in vitro* evaluation of their antioxidant properties is presented. Their effective role as antioxidants is influenced by their dual functions of reducing toxicity and inducing antioxidant effects.

Methods: Functionalization was confirmed by Fourier transform infrared spectroscopy (FTIR), and the total phenolic content was assessed. The antioxidant properties

* Corresponding address: Department of Nanoscience and Technology, School of Physical Sciences, Bharathiar University, Coimbatore, Tamil Nadu, 6410 46, India.
E-mail: rtrkumar@buc.edu.in (R.T. Rajendra Kumar)

Peer review under responsibility of Taibah University.

ELSEVIER Production and hosting by Elsevier

 http://dx.doi.org/10.1016/j.jtumed.2016.07.006

were analyzed by scavenging di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium, lipid peroxidation, reactive oxygen species (ROS) quantification and quenching externally generated hydroxyl and superoxide radicals.

Results: The functionalization of nanotubes with antioxidants was conformed via FTIR and measurement of total phenolic compounds. Higher radical scavenging was observed for TA-functionalized SWCNTs than for other functionalizations and MWCNTs. The lipid peroxidation results revealed that the functionalization of nanotubes with the antioxidant TA significantly decreased lipid peroxidation (36%) compared with naked nanotubes (85%) and the positive control (94%). Furthermore, antioxidant-functionalized nanotubes showed negligible production of ROS after being irradiated under different conditions, and externally generated hydroxyl and superoxide radicals were quenched.

Conclusion: This study showed, using *in vitro* models, that effective functionalization of CNTs with TA and GA leads to remarkable antioxidant properties. Antioxidant-functionalized nanotubes showed a reduction in cell lethality correlated with negligible ROS production under different irradiation conditions and quenching of externally generated hydroxyl and superoxide radicals. Further, antioxidant-functionalized nanotubes were more compatible with the cell membrane.

Keywords: Antioxidants; Biocompatibility; Carbon nanotubes; Functionalization; Radical scavenging

Introduction

Carbon-based nanomaterials, especially carbon nanotubes (CNTs), have opened new doors for a wide range of biological applications. Because of their unique electronic, chemical and mechanical properties, CNTs have potential applications in biological systems, including as biosensors,[1] biofilms,[2] drug carriers[3] and vaccine delivery vehicles.[4] Published reports show that application of CNTs in living system appears to be an elusive goal, as they have been found to be toxic and incompatible with biological systems.[5] Relevant to their toxicity, CNTs are oxidants that produce an excess of reactive oxygen species (ROS), causing deleterious effects, such as destruction of biological cell membranes and adenosine triphosphate and deoxyribonucleic acid fragmentation.[6,7] Studies have shown that purified single-walled carbon nanotubes (SWCNTs) were toxic to the PC12 cell line, directly or indirectly destroying cellular integrity and affecting gene regulation and cellular signaling pathways.[8,9] Further, time- and dose-dependent adverse effects on cell viability, morphological changes, DNA damage, increased apoptosis and damaged redox homeostasis have also been reported for CNTs with different functionalizations.[10,11] The available literature on the state of the art of CNTs reveals that their metal catalyst, mass, surface chemistry, purity and aspect ratio have a strong impact on their nature of toxicity.[5,6,12] However, from a biological perspective, functionalized CNTs are more compatible with the cell environment than naked ones. For instance, upon functionalization, CNTs were rendered biocompatible by inducing the formation of collagen in osteoblast cultures.[13] Functionalized CNTs favored ideal bone regeneration through mesenchymal stem cell proliferation *in vitro*.[5] Thus, targeted functionalization reduces the toxic nature of CNTs and favors their use in biological materials for multiple applications.[6]

To date, very few reports exist on the functionalization of CNTs with antioxidants. As antioxidants are effective radical scavengers of reactive oxygen species (ROS), their incorporation might reduce CNT toxicity. Recently, Lucente-Schultz, R. M. et al.[14] investigated antioxidant properties of SWCNTs functionalized with butylated hydroxytoluene (BHT). Follow-up studies showed that gallic acid (GA) functionalization of MWCNTs improved their free radical scavenging ability.[15] Lin, D. and Xing, B.[16] functionalized MWCNTs (multiwalled carbon nanotubes) with tannic acid (TA) for better dispersion.

In the present study, an attempt was made to functionalize the surface of SWCNTs and MWCNTs with water-soluble antioxidants, such as GA or TA, by a simple chemical approach. Our investigation revealed that naked CNTs were effective ROS generators, but that oxyradical generation was greatly suppressed in TA-/GA-functionalized ones. Further, through various *in vitro* assessments, such as measurement of the phenolic content, di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) assay, lipid peroxidation, and superoxide and hydroxyl radical scavenging activities, we found that TA-functionalized SWCNTs and MWCNTs had higher antioxidant properties than GA-functionalized and naked CNTs. These results may open a new area in a wide range of future biomedical applications.

Experimental

Antioxidant functionalization

Information regarding CNTs and the chemicals used for experiments is available at S1 in Appendix A. For antixodiant functionalization, a suspension of CNTs (1 mg/mL) and antioxidant (TA or GA) (1 mg/mL) was incubated for 3 days on a shaker at 120 rpm.[16] The resulting mixture was then sonicated (Rivotek, Mumbai, India) for 1 h and centrifuged at 3000 rpm for 15 min to remove excess unreacted antioxidants using a poly(tetrafluoroethylene) membrane filter (pore size: 0.2 μm). The filtered membranes were dried in oven at 80 °C for 24 h, and the dried samples then collected. The dried samples were re-dispersed (1 mg/mL) in water for antioxidant evaluation. They were then subjected to further analysis and assays. The antioxidant functionalization was assessed using Fourier transform infrared (FTIR) spectroscopy (Bruker, Tensor 47) in the range of 2000−700 cm^{-1}.

Estimation of total phenolic content

Antioxidant (TA or GA) functionalization on the nanotube surface was evaluated by estimating the presence of total phenolic compounds present on the antioxidant-functionalized CNTs. The total phenolic content of naked and antioxidant-functionalized CNTs was determined according to the method described by Siddhuraju and Becker.[17] Fifty-microliter aliquots of CNTs (1 mg/mL) were transferred to test tubes and diluted with distilled water to 1 mL. Then, 0.5 mL of Folin-Ciocalteu reagent (1:1 with distilled water) and 2.5 mL of sodium carbonate solution (20%) were added sequentially to each tube. Soon after vortexing the reaction mixture, the test tubes were incubated in the dark for 40 min, and absorbance was recorded at 725 nm against the reagent blank. The amount of phenolic contents in the sample was estimated in terms of gallic acid equivalent (GAE) or tannic acid equivalent (TAE).

Evaluation of antioxidant activity

Radical scavenging ability

The antioxidant activity of antioxidant (TA and GA)-functionalized and naked CNTs was determined in terms of their ability to donate hydrogens to the stable radical di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH), according to the method reported by Blois Marsden et al.[18] Naked and antioxidant (TA and GA)-functionalized CNTs (200 µl or 200 µg) were added to 2 mL of a 0.1 mM ethanolic solution of DPPH and shaken vigorously. The tubes were allowed to stand for 20 min at 27 °C. The absorbance of the sample solution was spectroscopically measured at 517 nm. The radical scavenging activity was calculated as the percentage of radicals using the Formula (1):

$$\text{Scavenging}(\%) = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100 \qquad (1)$$

Lipid peroxidation induced by CNTs

The efficacy of the CNT samples' induction of lipid peroxidation was assessed by a procedure reported by Ohkawa et al. with some modifications.[19] In the experimental procedure, goat liver was washed thoroughly in cold phosphate buffered saline (pH 7.4) and homogenized (Sonics and Materials, USA) to yield a 10% w/v homogenate. Liver cells are good indicators for estimating oxidative stress via the production of malondialdehyde formed through the peroxidation of their

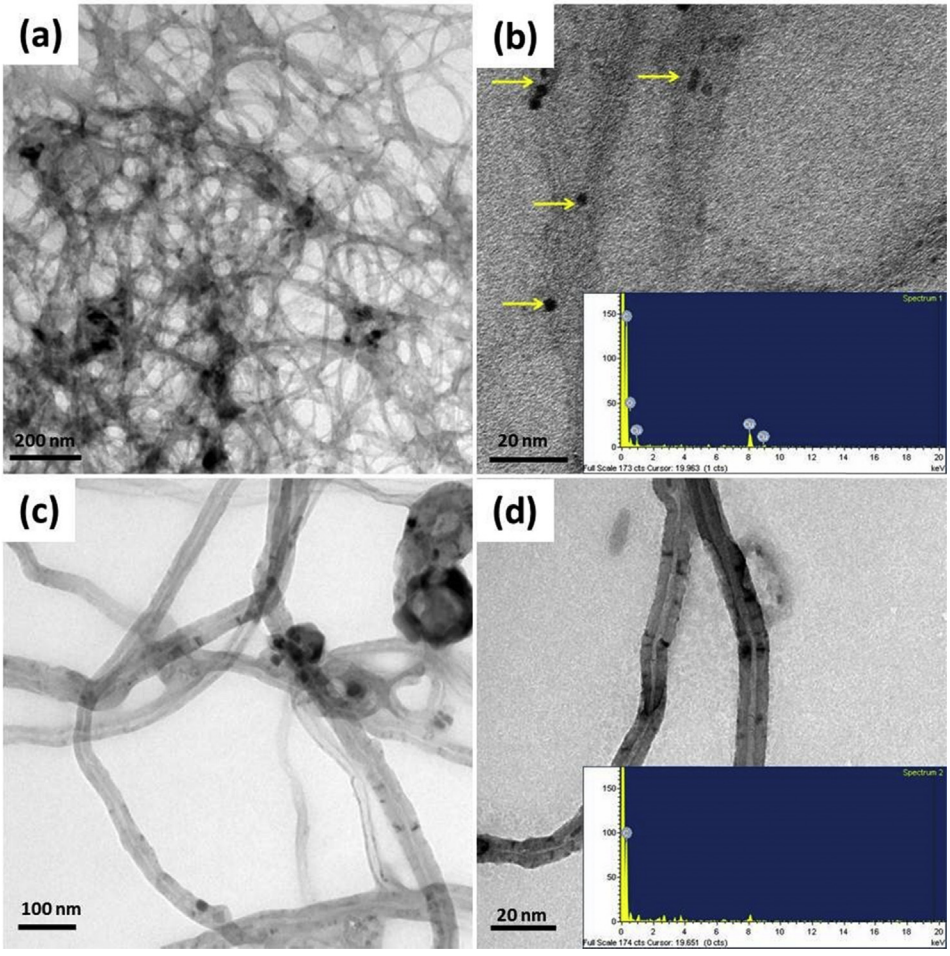

Figure 1: TEM images of purified long-length SWCNTs and MWCNTs. (a) Bundles of SWCNTs. (b) Individual networks of SWCNTs with trace amounts of amorphous carbon. (c) Purified MWCNTs. (d) Multiple stacked layers in MWCNTs. Inset of (b) and (d) are EDS spectra of SWCNTs and MWCNTs.

cell membrane. The detailed experimental procedure is available in Appendix A.

Reactive oxygen species generation estimation

The ability of CNTs (SWCNTs and MWCNTs) and antioxidant-functionalized CNTs (TA-SWCNTs and TA-MWCNTs) to produce reactive oxygen species (ROS) via different photo-induced reactions in solution was measured as described in a previous report.[7] The production of singlet oxygen ($^1O_2$) and hydroxyl radicals ($^{\bullet}OH$) was monitored by measuring the loss of furfuryl alcohol (FFA) (0.2 M) and *p*-chlorobenzoic acid (*p*CBA) (2 µM), respectively, in a 100-µl CNT (SWCNTs, MWCNTs, TA-SWCNTs and TA-MWCNTs) suspension incubated in direct sunlight (903 lm/m^2), in ambient light (180.6 lm/m^2) under laboratory conditions and in the dark. The light intensity was measured using a TES 1332 Digital lux meter. The superoxide radicals ($O_2^{\bullet-}$) formed were measured using 100 µl of CNTs in 50 mL of a nitroblue tetrazolium salt solution (NBT) (0.2 mM) that were exposed to the above light/dark conditions. In control tubes, the CNT suspension was replaced with distilled water. The samples were withdrawn at different time intervals (0.1, 0.3, 1, 2, 3 and 4 h) and passed through a 0.2-µm polytetrafluroethylene membrane filter, and the OD values were determined at 217, 237 and 530 nm for FFA, pCBA and NBT, respectively.

Superoxide ($O_2^{\bullet-}$) and hydroxyl ($^{\bullet}OH$) radical scavenging activity

The *in vitro* superoxide and hydroxyl radical scavenging activity of the antioxidant (TA or GA)-functionalized CNTs was assessed following standard procedures. The scavenging of superoxide radicals was measured according to the method reported by Beauchamp and Fridovich.[20] The detailed experimental procedure used for the quantification of superoxide and hydroxyl radical scavenging activity is given in S3 in Appendix A.

Results

Material characterization

Figure 1(a) shows transmission electron microscopy (TEM) images of bundles of SWCNTs as a network (see Supportive Information S1 for the experimental procedure). A uniform diameter and length distribution of SWCNTs are observed from the TEM images. Figure 1(b) shows high resolution TEM images of SWCNTs that contain trace amount of amorphous carbon (indicated with arrows in Figure 1(b)). The length and diameter of SWCNTs obtained from the TEM images were found to be in the range of 0.4–1.2 µm and 0.9–1.2 nm, respectively. The absence of metal impurities is supported by the recorded energy dispersive spectrum (EDS) of SWCNTs, and the inset image in Figure 1(b) reveals the purity of SWCNTs. TEM images of MWCNTs with multiple well-organized stacked layers are displayed in Figure 1(c). Higher magnification images of individual MWCNTs with a uniform wall thickness are observed in higher resolution TEM images (Figure 1(d)). The MWCNTs had different diameters as well as multiple stacked layers ranging from 10 to 50 nm (Figure 1(d)). The length distribution of MWCNTs is in the range of 0.5–1.5 µm. The absence of metal impurities is confirmed by the recorded EDS spectrum of MWCNTs (inset images of Figure 1(d)). Detailed material characterization of the nanotubes was reported previously.[7]

Fourier transform infrared spectroscopy

FTIR spectroscopy is used to ensure the functionalization of both SWCNTs and MWCNTs with the natural

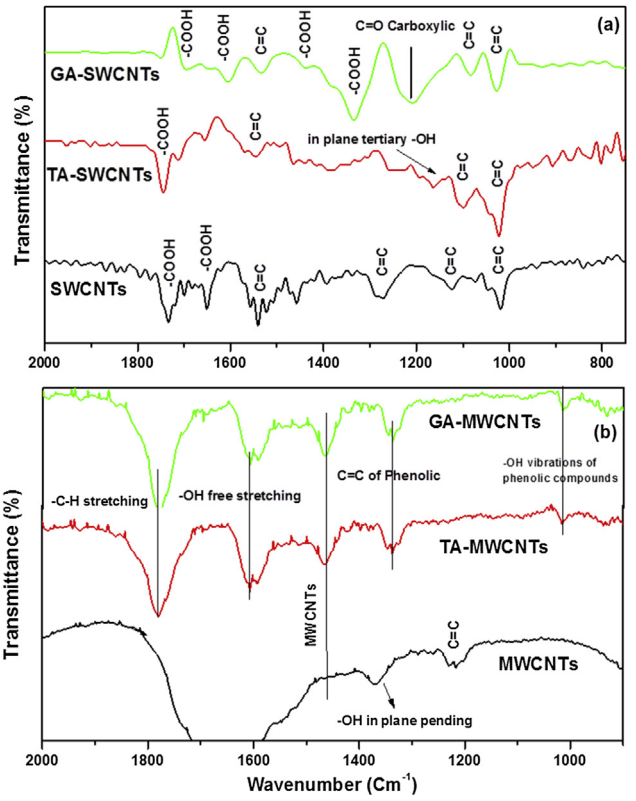

Figure 2: FTIR spectra of TA- and GA-functionalized (a) SWCNTs and (b) MWCNTs.

Table 1: Total phenolic contents of antioxidant-functionalized and naked CNTs.

Sample	Phenolic content	
	mg TAE/g CNT	mg GAE/g CNT
SWCNTs	–	–
MWCNTs	–	–
SWCNTs + GA	48.13 ± 3.2^c	52.86 ± 1.3^c
MWCNTs + GA	27.39 ± 1.7 ^{b}	31.57 ± 0.9^a
SWCNTs + TA	59.77 ± 3.0^c	56.13 ± 1.1 ^{b}
MWCNTs + TA	40.77 ± 1.2^a	37.12 ± 0.5^a

Values are expressed as the mean ± standard deviation (n = 3). The mean values are followed by different superscript letters in a column, indicating significant difference ($p < 0.05$), where a > b > c. The phenolic content is expressed as TA/GA equivalents per gram CNTs.

antioxidants TA and GA. Figure 2(a and b) shows the FTIR transmittance spectrum of TA- and GA-functionalized CNTs (SWCNTs and MWCNTs). The peaks observed in the fingerprint region for both TA- and GA-functionalized CNTs (SWCNTs and MWCNTs) confirm the attachment of antioxidants to the surface of CNTs. The observed peaks in Figure 2(a) in the fingerprint region at 1733, 1650, 1540, 1278, 1125 and 1018 cm^{-1} represent carboxylic acid functionalization on SWCNTs. These peaks are observed for both TA- and GA-functionalized SWCNTs. In addition, the vibration modes observed at 1745, 1542, 1614, 1344, 1224 and 1098 cm^{-1} are, respectively, of C=O stretching, aromatic C−H deformation, in-plane tertiary −OH bending and C=O stretching of carboxylic functional groups in antioxidant (TA or GA)-functionalized SWCNTs.[21,22] The peaks in Figure 2(b) observed at 1739, 1633 and 1354 cm^{-1} for MWCNTs are attributed to the C=C stretching vibration of aromatic rings of the rolled graphene sheet.[23] In addition, the peaks observed for MWCNTs at 3527, 3500, 2927, 1739, 1533 and 1367 cm^{-1} depict the free stretching of −OH stretching, −C−H stretching, $-CH_2$ vibration, −OH in-plane bending and C=O stretching and the −OH vibration of phenolic compounds, respectively.[24] Furthermore, the vibrations observed at 1217 cm^{-1} correspond to the −OH vibrations of phenolic compounds, collectively ensuring effective adsorption of the antioxidants (TA and GA) by MWCNTs.[24]

Total phenolic compound of functionalized CNTs

The effective attachment of antioxidants to functionalized CNTs (SWCNTs and MWCNTs) was further assessed from the total phenolic compounds measured using the Folin-Ciocalteu reagent.[25] The surface functionalization of CNTs with phenolic antioxidants GA or TA was further quantified by employing the Folin phenol assay, and the results are presented in Table 1. TA-functionalized SWCNTs show a higher percentage in the Folin phenol assay than TA-functionalized MWCNTs and have a higher calculated TAE. Similarly, GA-SWCNTs show significantly higher phenolic compounds than GA-MWCNTs, with a higher GAE. The effective attachment of antioxidants is more pronounced for SWCNTs than for MWCNTs. By contrast, these compounds were not detected on naked CNTs (SWCNTs and MWCNTs).

Antioxidant properties of functionalized CNTs

DPPH• scavenging activity of antioxidant-functionalized CNTs

The radical scavenging potential of antioxidant-functionalized CNTs was quantified using the DPPH• radical scavenging assay. The percent of DPPH radical scavenging activity for different functionalized CNTs was studied, and the results are presented in Figure 3(a). From the observed results, it is evident that antioxidant-functionalized CNTs showed higher DPPH• scavenging activity than naked CNTs. SWCNTs were found to have significantly higher radical scavenging activity (56.23% and 57.43% for TA and GA functionalization, respectively) than MWCNTs (49.71% and 45.86% for TA and GA functionalization, respectively). Furthermore, the higher DPPH radical scavenging potential of antioxidant (TA)-functionalized SWCNTs may be attributed to the higher quantities of antioxidants adsorbed to the surface in comparison with the amounts adsorbed to MWCNTs (Figure 3(a)), in agreement with an earlier report.[15]

Lipid peroxidation

The *in vitro* lipid peroxidation ability of antioxidant-functionalized CNTs (SWCNT and MWCNTs) was quantified by measuring the lipid peroxidation contents using the formation of malondialdehyde. Figure 3(b) shows the amounts excreted in the lipid peroxidation process of antioxidant-functionalized CNTs. It is interesting to note that both naked CNTs show strong lipid peroxidation of lipid bilayers (85% and 83% for SWCNTs and MWCNTs, respectively) that is close to that of the positive control H_2O_2

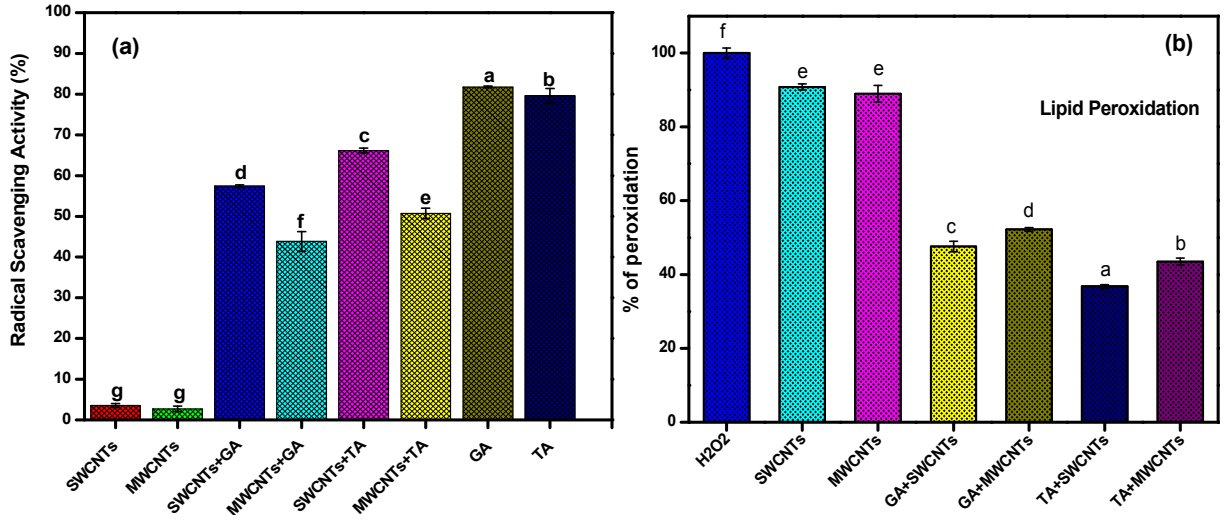

Figure 3: (a) DPPH radical scavenging activity of naked and antioxidant (TA or GA)-functionalized CNTs (SWCNT and MWCNTs). (b) Malondialdehyde contents produced upon treatment of goat liver cells with naked and antioxidant (TA or GA)-functionalized SWCNTs and MWCNTs (100 μg/mL).

(94%). However, the decrease in lipid peroxidation upon antioxidant (TA or GA) functionalization is significant. TA-functionalized CNTs (SWCNTs: 36% and MWCNTs: 43%) show less lethality to lipid layers than GA-functionalized CNTs (SWCNTs: 47% and MWCNTs: 52%).

Estimation of ROS generation

The radical generation capability of naked and TA-functionalized CNTs (SWCNTs and MWCNTs) was analyzed using different radicals ($^1O_2$, ($O_2^{\bullet -}$) and $^{\bullet}OH$) quencher compounds (FFA, NBT and pCBA), and the results are presented in Figure 4 (a, b and c, respectively). From the radical scavenging test, naked SWCNTs and MWCNTs were found to be effective generators of radical species ($^1O_2$, ($O_2^{\bullet -}$) and $^{\bullet}OH$), as evidenced by an increase in absorbance due to the reduction of FFA, NBT and pCBA contents. In addition, the antioxidant (TA)-functionalized CNTs (SWCNTs and MWCNTs) were observed to produce negligible ROS, with a lower absorbance value due to the reduction of FFA, NBT and pCBA, similar to the results of dark exposure. These results reveal that antioxidant-functionalized CNTs suppress the production of radical species ($^1O_2$, ($O_2^{\bullet -}$) and $^{\bullet}OH$), even at high irradiation (903 lm/m^2).

$O_2^{\bullet -}$ and $^{\bullet}OH$ radical scavenging activity

$O_2^{\bullet -}$ and $^{\bullet}OH$ radicals are highly reactive, toxic radical species that are implicated in several deleterious processes,

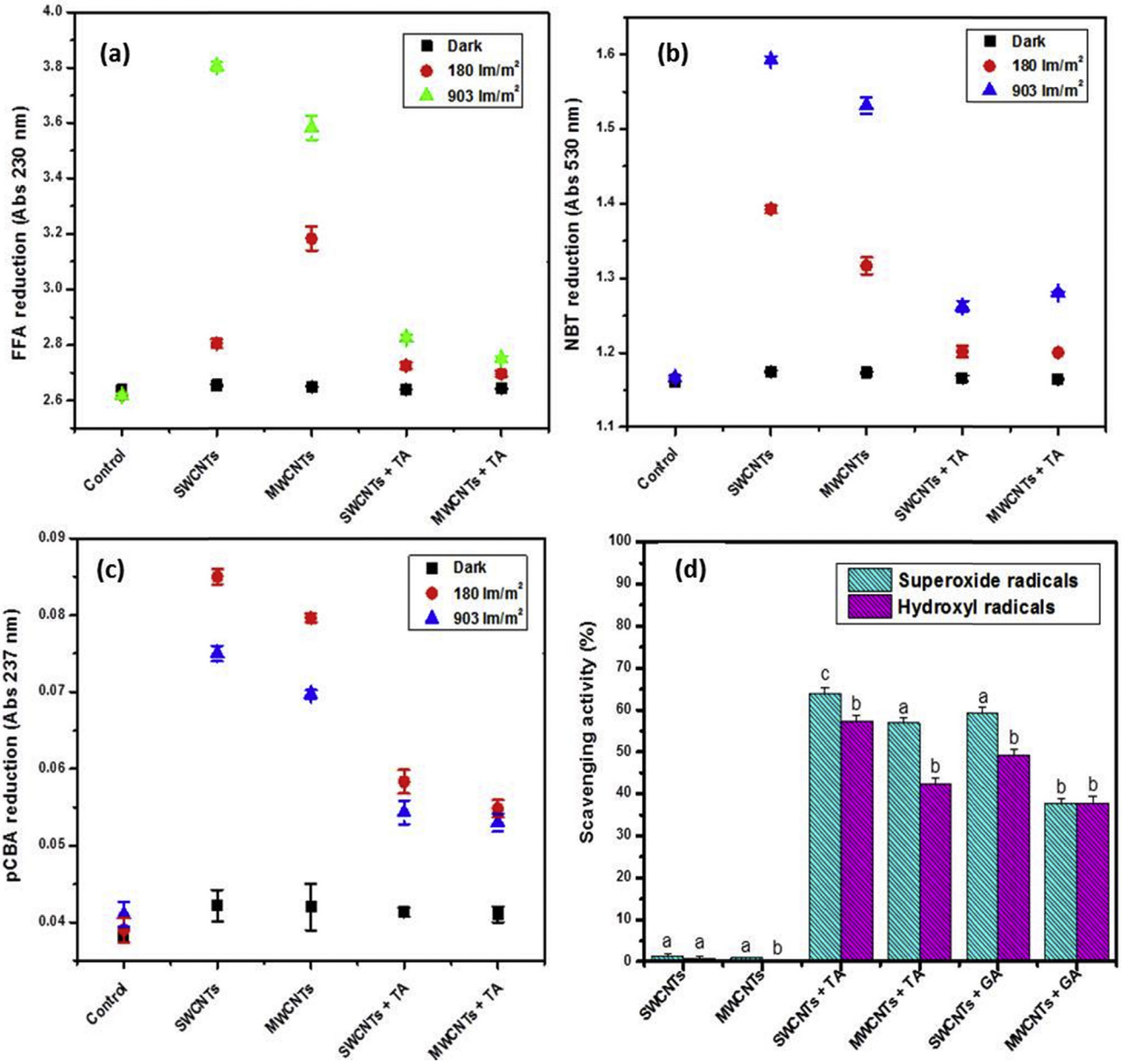

Figure 4: Production of ROS by CNT suspension after different times of exposure to direct sunlight (903 lm/m^2), ambient light under laboratory conditions (180 lm/m^2) and dark (a) for FFA reduction, (b) for NBT reduction and (c) for pCBA reduction. (d) Super oxide ($O_2^{\bullet -}$) and hydroxyl ($^{\bullet}OH$) radical scavenging activities of naked and antioxidant (TA or GA)-functionalized CNTs (100 μg/mL).

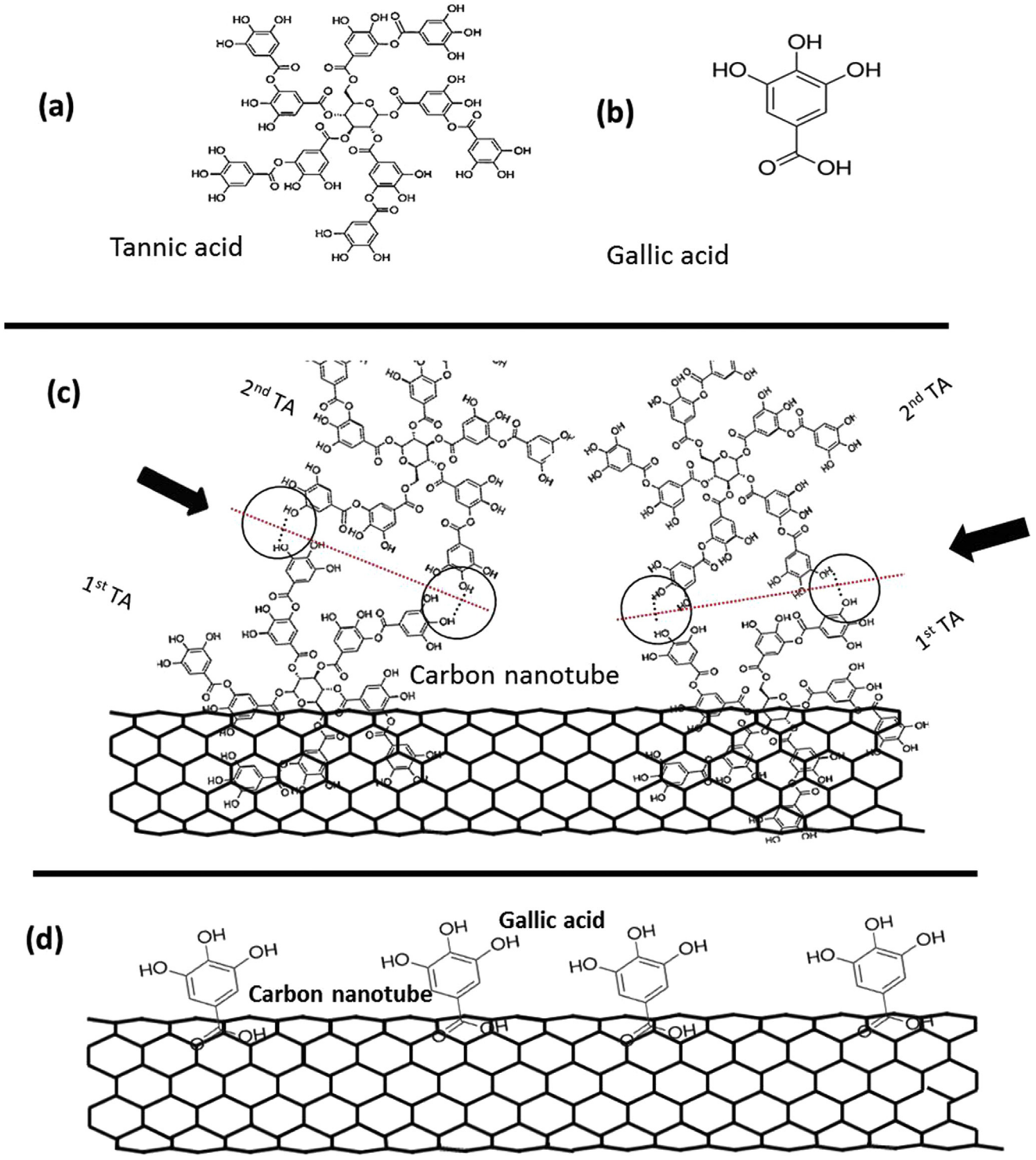

Figure 5: Schematic representation of interaction between CNTs and antioxidants, where (a) and (b) are the molecular structure of TA and GA, respectively. (c) Anchoring of TA to the surface of CNTs via π-π interactions to form a primary layer (indicated as "1st TA molecule") followed by the secondary layer (indicated as "2nd TA molecule"). (d) Gallic acid interacts with the surface of CNTs to form a monolayer with minimal hydroxyl groups.

such as lipid peroxidation and damage to protein and DNA molecules in living cells. Therefore, an experiment was set up to assess the ability of antioxidant-functionalized CNTs to scavenge externally generated $O_2^{\bullet-}$ and $^{\bullet}OH$ radicals. Therefore, superoxide was generated using the nitroblue tetrazolium-riboflavin method, whereas $^{\bullet}OH$ was produced in an ascorbic acid-iron-ethylenediaminetetraacetic acid system. The scavenging ability of naked and antioxidant-functionalized CNTs was analyzed, and the results are presented in Figure 5. The results presented in Figure 5(d) show remarkable $O_2^{\bullet-}$ and $^{\bullet}OH$ radical scavenging activities in the antioxidant-functionalized CNT (SWCNT and MWCNT) suspension. SWCNTs functionalized with antioxidants were indeed again more effective radical scavengers than antioxidant-functionalized MWCNTs. A more remarkable quenching effect was observed for TA-CNTs (SWCNTs and MWCNTs) than for GA-CNTs. On the other hand, naked CNTs failed to show any detectable radical scavenging activity in an aqueous suspension. The radical scavenging activity results correlate well with the results of the lipid peroxidation and DPPH radical scavenging assays.

Discussion

Suitable functionalization with selective organic frameworks has become more important in the production of biocompatible nanotubes.[2,3,5] Effective functionalization with an antioxidant is a potential method to both effect a reduction in toxicity and induce antioxidant benefits. The influence of molecular structures on functionalization and antioxidant properties was analyzed with naked and TA-/GA-functionalized CNTs. The differential efficacy of CNT functionalization with antioxidants (TA or GA) could be related to their molecular structure and the efficiency of their binding to the surface of CNTs (Figure 5(a–d)). TA binds easily to CNTs, as it contains more aromatic rings and free hydroxyl groups than GA (Figure 5(a and b)).[26] The sorption of TA onto the walls of CNTs may begin with anchoring to the surface of the carbon rings of CNTs via π-π interactions due to their π-donor and π-acceptor characters (Figure 5(c), showing the binding of the first TA molecule). The successful attachment, with different bonds formed in the process, of antioxidants to different nanotubes is evidenced by the observed fingerprint vibrations in the FTIR spectra as well as by the measured total phenolic contents (Figure 2 and Table 1). To estimate the amount of phenolic derivatives in antioxidant-functionalized CNTs, the DPPH radical assay was performed, revealing that TA-functionalized SWCNTs are effective in quenching DPPH radicals compared with other functionalized and naked CNTs (Figure 3(a)). The mechanism by which CNTs exposed to biological media are toxic is reported to be through a series of chemical reactions, such as generation of ROS, followed by oxidative stress on the cell membrane, resulting in severe cell damage.[7,14,27] Thus, *in vitro* cell lethality at the lipid bilayer was analyzed via the lipid peroxidation assay by measuring malondialdehyde with a positive control (H_2O_2). The one-fold decrease in lipid membrane breakage by antioxidant functionalization is more interesting for active biocompatible nanotubes (Figure 3(b)). The lipid peroxidation of cell membranes is generally caused by excessive oxidative stress on cell membranes.[7] It has been reported that radical species are an effective cause of oxidative stress on the cell membrane. To analyze oxidative stress on the cell membrane, experiments were performed to quantify reactive oxygen species (ROS). It is interesting to note that naked CNTs are effective generators of radicals (Figure 4(a–c)), while antioxidant-functionalized CNTs fail to produce radical species under different irradiation conditions. Further, the ability of antioxidant-functionalized CNTs to scavenge externally generated radical species ($O_2^{\bullet-}$ and $^{\bullet}OH$) was further evaluated by radical scavenging assays. It is obvious from the results presented in Figure 4(d) that functionalization of CNTs with TA rendered them more efficient scavengers of free radicals than functionalization with GA. Furthermore, TA-functionalized CNTs possess more free hydroxyl groups than GA and were found to be more effective quenchers of radical species.[28] Thus, the peculiar structural features of TA-functionalized CNTs rendered them more favorable in antioxidant properties than GA-functionalized CNTs in *in vitro* assays. Additionally, as our quantitative experiments from different *in vitro* models suggest, the antioxidant-functionalized SWCNTs were more compatible with the cell membrane, and this can be used as an effective method to make biocompatible materials for biomedical applications.

Conclusion

A simple, cost-effective methodology was effectively applied for antioxidant functionalization of CNTs (SWCNTs and MWCNTs). The present study shows that CNTs are toxic to living cells due to their ability to initiate lipid peroxidation, resulting in membrane damage. However, upon functionalization with the antioxidant TA or GA, the oxidizing ability of CNTs was found to be considerably reduced. Assessment of lipid peroxidation using goat liver cells indicated that the membrane damage was severe in naked CNTs but was significantly suppressed upon antioxidant functionalization. Antioxidant-functionalized CNTs were able to neutralize the free radicals generated *in vitro* and retarded lipid peroxidation. The present study shows that the toxicity of naked CNTs can be toned down by functionalizing them with antioxidants, which will enhance the scope of biomedical applications of CNTs.

Authors' contributions

SM and RTR planned the work, KR and RG conducted the experiments. RTR interpreted data and wrote the final draft of the article. All of the authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgments

One of the authors, KR, is grateful to CSIR, New Delhi for the award of SRF.

Appendix B. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jtumed.2016.07.006.

References

1. Vo-Dinh T, Cullum B. Biosensors and bio chips: advances in biological and medical diagnostics. **Fresenius J Anal Chem 2007**; 366: 540–551.
2. Rodrigues DF, Elimelech M. Toxic effects of single-walled carbon nanotubes in the development of *E. coli* biofilm. **Environ Sci Technol 2010**; 44: 4583–4589.
3. Bianco A, Kostarelos K, Prato M. Applications of carbon nanotubes in drug delivery. **Curr Opin Chem Biol 2005**; 9: 674–679.
4. Pantarotto D, Briand JP, Prato M, Bianco A. Translocation of bioactive peptides across cell membranes by carbon nanotubes. **Chem Commun 2004**; 1: 16–17.
5. Liu D, Yi C, Zhang D, Zhang J, Yang M. Inhibition of proliferation and differentiation of mesenchymal stem cells by carboxylated carbon nanotubes. **ACS Nano 2010**; 4: 2185–2195.
6. Joshi A, Punyani S, Bale SS, Yang H, Borca-Tasciuc T, Kane RS. Nanotube-assisted protein deactivation. **Nat Nanotechnol 2008**; 3: 41–45.
7. Rajavel K, Gomathi R, Manian S, Rajendrakumar RT. In vitro bacterial cytotoxicity of CNTs: reactive oxygen species mediate cell damage edges over direct physical puncturing. **Langmuir 2014**; 30: 592–601.
8. Wang J, Sun P, Bao Y, Liu J. Cytotoxicity of single-walled carbon nanotubes on PC12 cells. **Toxicol Vitro 2011**; 25: 242–250.
9. Prabakaran R, Sudhakar B, Bindhu S, Gopikrishnan R, Biradar S, Ramesh V, Hall JC, Ramesh GT. Multiwalled carbon nanotubes activate NF-jB and AP-1 signaling pathways to induce apoptosis in rat lung epithelial cells. **Apoptosis 2010**; 15: 1507–1516.
10. Taheramnsouri H, Mirosanloo A, Keshel SH, Gardanes M. Synthesis, characterization and toxicity of Multiwalled carbon nanotubes functionalized with 4-hydroxyquiazoline. **Carbon Lett 2016**; 17: 45–52.
11. Tahermansouri H, Abedi E, Heidari-Keshel S, Tarlani A. Influence of functionalized multiwalled carbon nanotubes with imidazole derivative and thiosemicarbazide on MHN45 and SW742 cancer cells. **Mater Technol Adv Perform Mater 2015**; 30: 223–229.
12. Pasquini LM, Hashmi SM, Sommer TJ, Elimelech M. Impact of surface functionalization on bacterial cytotoxicity of single-walled carbon nanotubes. **Environ Sci Technol 2012**; 46: 6297–6305.
13. Zanello LP, Zhao B, Hu H, Haddon RC. Bone cell proliferation on carbon nanotubes. **Nano Lett 2006**; 6: 562–567.
14. Lucente-Schultz RM, Moore VC, Lenonard AD, Price BK, Kosynkin DV, Lu M, Partha R, Convyers JL, Tour JM. Antioxidant single-walled carbon nanotubes. **J Am Chem Soc 2009**; 131: 3934–3941.
15. Cirillo G, Hampel S, Klingeler R, Puoci F, Iemma F, Curcio M, Parisi OI, Spizzirri UG, Picci N, Leonhardt A, Ritschel M, Büchner B. Antioxidant multi-walled carbon nanotubes by free radical grafting of gallic acid: new materials for biomedical applications. **J Pharm Pharmacol 2011**; 63: 179–188.
16. Lin D, Xing B. Tannic acid adsorption and its role for stabilizing carbon nanotube suspensions. **Environ Sci Technol 2008**; 42: 5917–5923.
17. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. **J Agric Food Chem 2003**; 51: 2144–2155.
18. Blois MS. Antioxidant determinations by the use of a stable free radical. **Nature 1958**; 181: 1199–1200.
19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. **Anal Biochem 1979**; 95: 351–358.
20. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. **Anal Biochem 1971**; 44: 276–287.
21. Wang YB, Iqbal Z, Mitra S. Rapidly functionalized water-dispersed carbon nanotubes at high concentration. **J Am Chem Soc 2006**; 128: 95–99.
22. Ramanathan T, Fisher FT, Ruoff RS, Brinson LC. Amino-functionalized carbon nanotubes for binding to polymers and biological systems. **Chem Mater 2005**; 17: 1290–1295.
23. Arauji PZ, Morando PJ, Blesa MA. Interaction of catechol and gallic acid with titanium dioxide in aqueous suspensions. Equilibrium studies. **Langmuir 2005**; 21: 3470–3474.
24. Pantojacastro MA, Goznalezrodriguez H. Study by infrared spectroscopy and thermogravimetric analysis of tannins and tannic acid. **Rev Latinoam Quím 2011**; 39: 107–112.
25. Amarowicz P, Peggb RB, Rahimi-Moghaddam P, Barl B, Weil JA. Free-radical scavenging capacity antioxidant activity of selected plant species from the Canadian Prairie. **Food Chem 2004**; 84: 551–562.
26. Isenburg JC, Karamchandani NV, Simionescu DT, Vyavahare NR. Structural requirements for stabilization of vascular elastin by polyphenolic tannins. **Biomaterials 2006**; 27: 3645–3651.
27. Khan NS, Ahmad A, Hadi SM. Anti-oxidant, pro-oxidant properties of tannic acid and its binding to DNA. **Chem Biol Interact 2000**; 125: 177–189.
28. Halkes SB, Vrasidas I, Rooijer GR, Van Den Berg AJ, Liskamp RM, Pieters RJ. Synthesis and biological activity of polygalloyl-dendrimers as stable tannic acid mimics. **Bioorg Med Chem Lett 2002**; 12: 1567–1570.

How to cite this article: Rajavel K, Gomathi R, Manian S, Rajendra Kumar RT. Characterization of tannic acid- and gallic acid-functionalized single- and multiwalled carbon nanotubes and an *in vitro* evaluation of their antioxidant properties. J Taibah Univ Med Sc 2016;11(5):469–477.